The Embryology and Development Of *Strongylocentrotus purpuratus*

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Introduction:

Strongylocentrotus purpuratus was chosen as the subject of this paper because it is familiar in the adult form and it has interesting development. This animal survives well in laboratory conditions which is one reason it is extensively documented. Observations of the rearing of this organism, more specifically the development of the hydrocoel from the enterocoel, have been published since the 1880's by H. Bury's *Studies in the Embryology of Echinoderms* and more recently, the methods of rearing, by Megumi F. Strathman, 1987.

S. purpuratus are deuterostomes like humans. Their developing transparent embryo can be studied easily and without ethical implications. Its evolution is also well analyzed since the test survives easily in the fossil record. It is gonochoristic which means that each individual is either male or female. Nevertheless hermaphroditism is a 1 in 500 occurrence (Pearse and Cameron. 1991). Any beachcomber knows that an urchin is impossible to sex by its external characteristics. I have dissected this animal and when the test is opened, the gametes clearly show which sex the animal is. Eggs are yellow orange.

These animals have pentamerous radial symmetry. They have five radial nerves branching out from a ring canal. Their epidermis covers a hard test as well as stiff spines used for protection. They also have pedicilaria used for protection and keeping the exterior of the animal clean. There is an extensive complete digestive system with an anus on the dorsal side. Gonads are arranged in five sections because of its symmetry. The gonads have three epithelial layers: the perivisceral peritoneum, the muscular epithelium and the germinal epithelium (Pearse and Cameron. 1991). All these layers do not go through the gonopore in genital plate. It is the germinal epithelium that goes through the gonopore; the contractions of the muscular epithelium releases the gametes.

Environment plays a big role in determining the sexual seasons of *S. purpuratus*. . When species have been transplanted, their spawning has converged with the local population (Pearse and

Cameron. 1991). At high latitudes gametogenesis is short and late in the year while at lower latitudes it is early and for a longer period. Gametogenesis requires 17 to 12 degree Celsius conditions. Photoperiodism is observed affecting gametogenesis, which is initiated with short days and repressed by long days.

Broadcast spawning occurs in late winter and early spring on the Oregon coast. Fertilization occurs with at least 1 million sperm per liter. Sperms are active for less than 20 minutes (Pearse and Cameron. 1991). Pheromones are released with sperm which is thought to stimulate the spawning of eggs. The radial nerve is the actual site where spawning is stimulated.

Eggs that are spawned are surrounded by jelly. This jelly expands and hydrates when in contact with sea water. Sea water influences the metabolism of the sperm increasing their motility because of an influx of sodium ions and an efflux of hydrogen ions (Pearse and Cameron. 1991).

Methods

When we stimulated our specimens to spawn, we injected each individual several times with several milliliters of KCL through the peristomial membrane on the ventral side into the body cavity and the gonads or radial nerves. We collected the sperm and eggs for several minutes in dishes beneath the spawning embryos. Each of us took a pipette of eggs and sperm and observed them under the compound scope before mixing them with sea water in a dish. Before fertilization the animal vegetal axis is defined in the egg.

When a sperm contacts an egg there are some reactions before the sperm moves to the cytoplasm. There is the acrosomal reaction where the acrosomal granule in the sperm head is everted before the sperm is fused with the plasma membrane. Enzymes are released; these help the sperm move through the jelly coat. The influx of calcium facilitates the making of the fertilization envelope. There is a 25 seconds delay between contact with a sperm and the making of the fertilization envelope. During this time polyspermy can result. There is also a rise in membrane potential from -70mV to +10mV (Pearse and Cameron. 1991). This is the fast block to

polyspermy.

The sperm nucleus becomes the male pronucleus in three steps: 1. lamellae of nuclear membrane fuse, sperm chromatin is in contact with the cytoplasm 2. chromatin becomes the nucleoplasm 3. membrane-bound vesicles form the new nuclear envelope (Pearse and Cameron. 1991).

When the fertilized egg was observed it had two polar bodies extruded on the out side of the egg but inside the vittelline envelope. These polar bodies are the result of meiosis of the egg nucleus. The sperm must wait for meiosis. Cleavage occurs between mitotic asters and is most timely at 15 degrees Celcius. Four vegetal cells divide unequally - four micromeres and four macromeres result. From the animal blastomeres come the oral and aboral ectoderm and neurons of the early pluteus.

The timetable for attachment of sperm to first cleavage is summarized by Pearse and Cameron:

1. Sperm attachment
2. Calcium and sodium action potential
3. Gamete fusion
4. Sodium activation potential
5. Cortical reaction
6. Activation of NAD kinase
7. Increase in intracellular ph
8. Initiation of protein synthesis
9. Increase in Potasium conductance
10. Karyokinesis
11. Initiation of DNA synthesis
12. First cleavage

This summary is missing some steps like the extrusion of polar bodies. Many steps were not observed such as Activation of NAD kinase and increase in potassium conductance.

The dish with eggs and sperm were washed at the end of the day. A 50 micrometer screen was used to filter the water that was sucked out with a syringe. Initially the excess sperms were taken through the filter. In later days ciliates and bacteria were filtered away every two days. The dishes were kept in water table at 12 to 15 degrees C. Later the feeding larvae were fed microalgae.

Results

Cleavage was observed within an hour of fertilization. Telomeres could be seen very faintly. The eight cell stage was seen within hours of fertilization. After twenty four hours a blastocoel was observed. At fifty four hours invagination began. Secondary mesenchyme cells were clearly observable as the archenteron extended. At this time a spin in the invaginated blastocoel was observed. The method or reason for this is not known. At seventy two hours after fertilization triradiate spicules were clearly developing. These became fenestrated spicules. This is the beginning of the early pluteus stage. At one week a feeding larvae were observed injesting algae.

1 hour	Cleavage
2 ½ hours	4 cell stage (polar bodies seen)
5 hours	8 cell stage
24 hours	Blastocoel
55 hours	Invagination
72 hours	Triradiate spicules
96 hours	Early pluteus
1 week	Pluteus
3 weeks	Late pluteus with developing test
4 weeks	Juvenile

I observed a late pluteus with a small test shell or rudiment. It moved with the aid of two ciliary bands. The juvenile exhibits positive geotaxis when settling at the bottom of the dish.

Discussion

The results are comparable to Strathmann who presents a nice table with stages of development for three temperatures. Our temperatures varied from 12 to 15 degrees and our results are varied but were not outside the boundary of Strathmann's three temperatures.

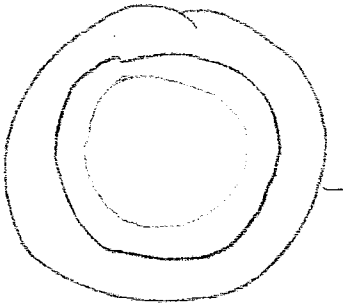
There was a problem with larvae sticking to the bottom of the filter. Other species were found in the *S. purpuratus* dish and *S. purpuratus* was found in other cultures. This was later resolved by pipetting each larvae out of the dirty dish and into clean sea water every two days. This would be too time consuming during the initial stage when there were at least 100 fertilized eggs. Care needs to be taken to ensure the filter is cleaned after screening each culture.

Literature cited:

Pearse, J.S. R.A. Cameron *Reproduction of Marine Invertebrates* Volume VI pgs. 513-662 1991.

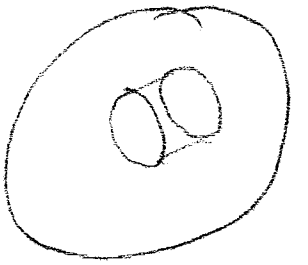
Strathmann, M.F. *Reproduction and Development of Marie Invertebrates of the Northern Pacific Coast* 1987.

Strongylocentrotus purpuratus

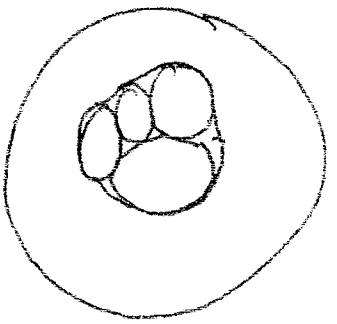


Fertilization

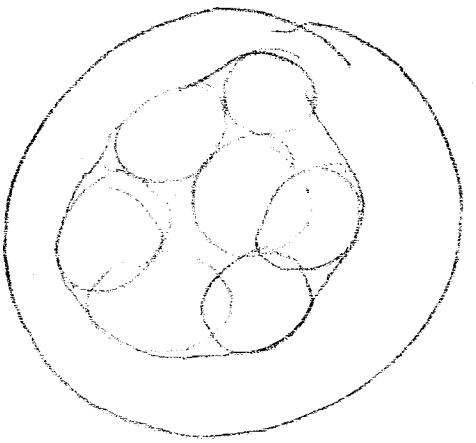
- vitelline envelope



Cleavage



Four cell stage



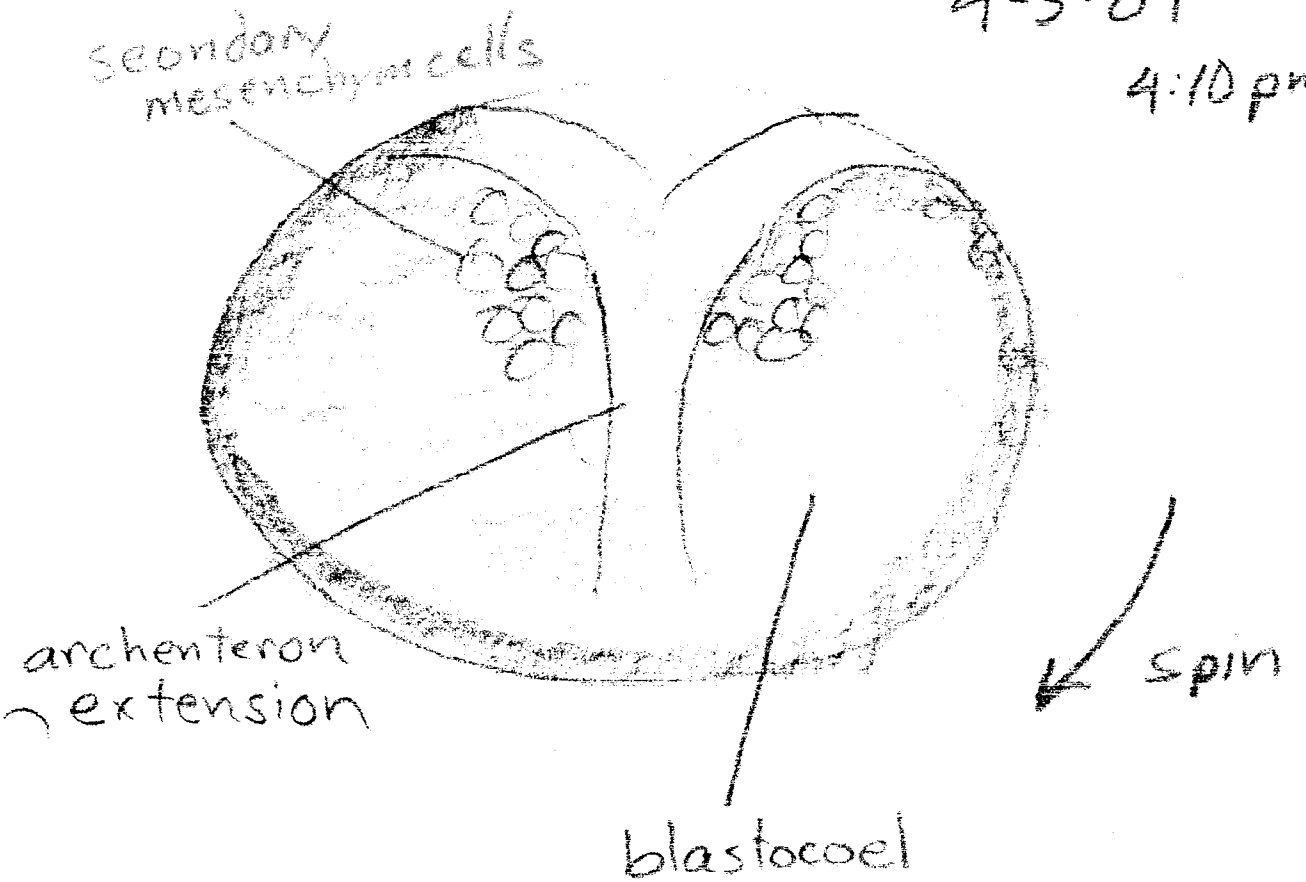
eight cell stage

Strongylocentrotus purpuratus

55 hrs

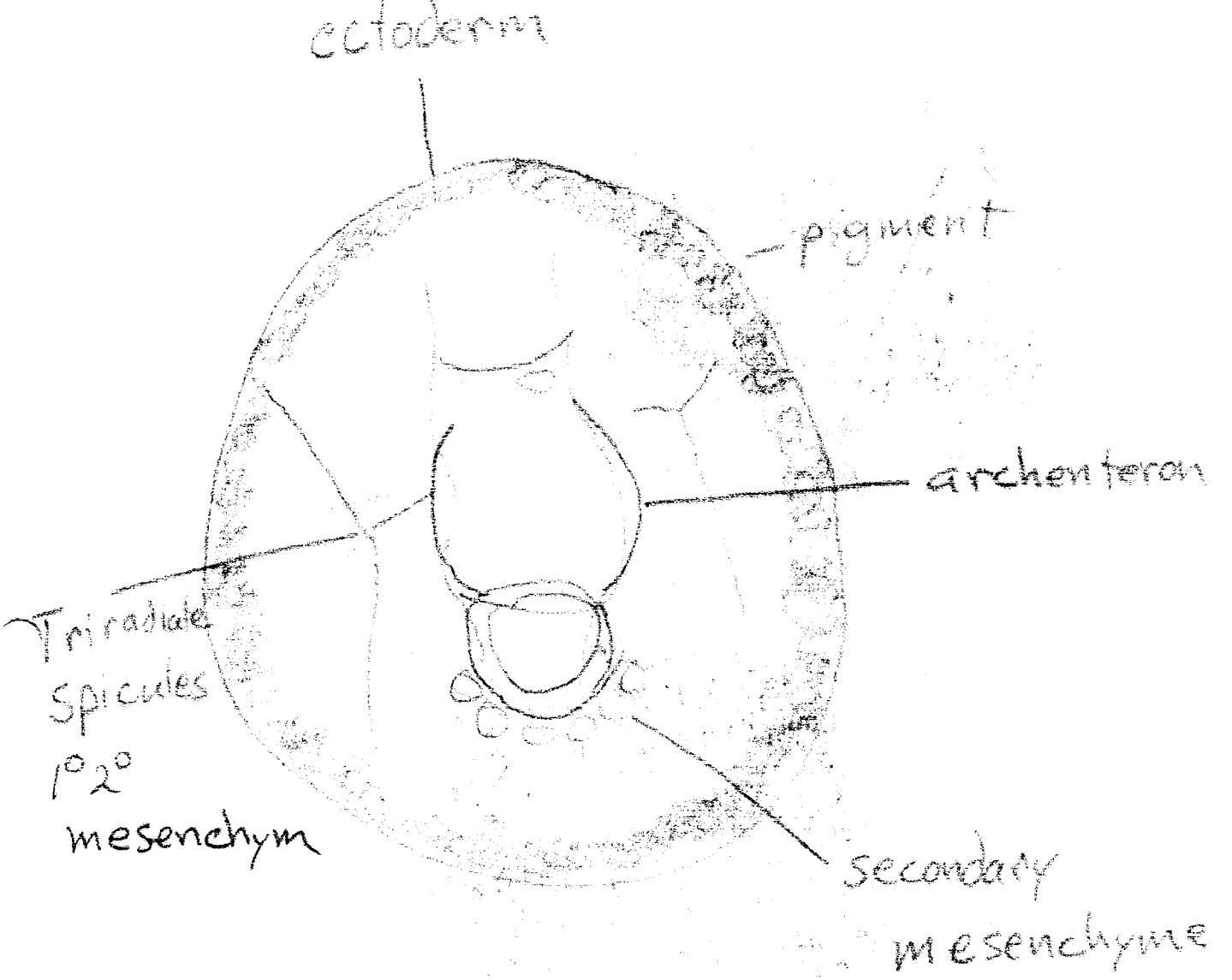
4-5-07

4:10 pm



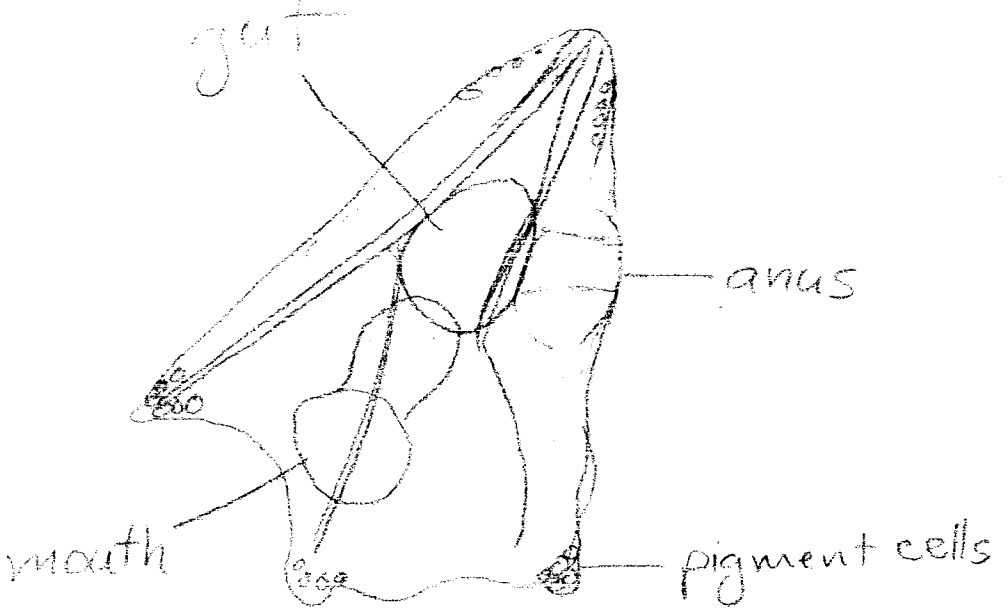
Strongylocentrotus purpuratus

4-6 72 hrs



Strongylocentrotus purpuratus

4 - 12



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late echinopluteus